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Award Number: DAMD17-97-1-7088

TITLE: Clonal Hematopoiesis as a Marker of Genetic Damage
Following Adjuvant Chemotherapy for Breast Cancer: Pilot
Study to Evaluate Incidence

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REPORT DATE: September 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 2000		3. REPORT TYPE AND DATES COVERED Annual (18 Aug 99 – 18 Aug 00)
4. TITLE AND SUBTITLE Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence			5. FUNDING NUMBERS DAMD17-97-1-7088	
6. AUTHOR(S) Charles A. Coltman, Jr., M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) CTRC Research Foundation San Antonio, Texas 78229-3264 E-MAIL: ccoltman@swog.org			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) A serious late effect complication associated with breast cancer treatment is the increased risk for development of therapy-related hematologic malignancies. The goal of this study is to determine whether dose-intensive adjuvant regimens for breast cancer induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis. Clonal hematopoiesis has been proposed as an early marker of hematopoietic stem cell damage, preceding the acquisition of critical, recurring genetic alterations associated with the development of therapy-related myelodysplastic syndromes and acute leukemia. Clonal hematopoiesis is being evaluated by two different methods, the X-linked HUMARA clonality and microsatellite instability assays. All positive results will be further analyzed for MLL and RAS alterations. Study accomplishments to date: a) activation of ancillary biological protocol (S9719) to treatment protocol (S9623), b) testing assays developed and standardized, c) specimen collection and data analysis of 123 samples from 21 patients completed, d) protocol amendment incorporating S9719 into the clinical treatment (S9623) protocol submitted and approved by DoD and CTEP to increase patient accrual, e) S9623/S9719 declared a high priority clinical trial by the NCI, f) S9719 protocol updates given at Southwest Oncology Group meetings, and g) presentation of data collected at the Era of Hope 2000 meeting.				
14. SUBJECT TERMS Breast Cancer				15. NUMBER OF PAGES 51
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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INTRODUCTION

The goal of this study is to determine whether dose-intensive adjuvant regimens for breast cancer induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis by studying sequential blood/bone marrow samples from 200 women enrolled in a single, randomized dose-intensive Southwest Oncology Group (SWOG) adjuvant breast cancer study for women with four or more positive nodes (S9623, "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with 4 or More Involved Axillary Lymph Nodes, Phase III, Intergroup"). Two different general clonality assays are used to detect clonality: the HUMARA (human androgen receptor) assay to estimate the incidence of early genetic damage defined by the presence of clonal hematopoiesis and microsatellite instability testing to screen for loss of heterozygosity or the presence of defective DNA mismatch repair mechanisms. In cases where either the HUMARA or microsatellite repeat assay is positive for clonality, the incidence of *MLL* fusion gene transcripts and *RAS* gene mutations (*H-RAS*, *K-RAS*, and *N-RAS*) will be performed.

BODY OF PROGRESS REPORT

Statement of Work, Problems Encountered In Accomplishing Study Objectives, Proposed Solutions.

The Southwest Oncology Group S9719 clonal hematopoiesis protocol was activated on October 15, 1997. Official notification to all Southwest Oncology Group member institutions occurred with the November 1, 1997 Group mailing. Drs. Slovak and Stock presented introductory protocol seminars to the Breast, Bone Marrow and Stem Cell Transplantation, and Leukemia Biology Committees at the 1997-1998 Southwest Oncology Group meetings. Despite the advertisements and protocol presentations, patient accrual was slower than anticipated causing concern for the inability to draw meaningful conclusions in the proposed timeframe. An October 1998 survey indicated two barriers: 1) many Southwest Oncology Group institutions did not open the biological study and were not offering S9623 patients the option due to short staffing of research nurses/clinical research associates; and 2) the requirement of two separate consent forms was cumbersome for medical professionals and their patients. To increase patient accrual and obtain the biological information needed to answer the question put forth by this research proposal, S9719 study coordinators submitted a request to the Department of Defense (DOD) and Cancer Therapy and Evaluation Program (CTEP) in 1999 to incorporate S9719 directly into the primary treatment protocol (S9623).

Amendments Approved in Year 2000. The amendment proposed: 1) Southwest Oncology Group will require concurrent registration to S9719 at the time of S9623 registration for Group institutions and 2) The Southwest Oncology Group will remove the model informed consent from S9719 and incorporate it into the S9623 model informed consent form. These changes addressed the concerns stated above for Southwest Oncology Group centers, medical professionals, and their patients. The amendments also ensure S9719 is offered to patients at the time of S9623 registration. Although the consent forms are consolidated into one form, patients always have the option to participate in the clinical protocol without the requirement of participating in the biological study. We are optimistic that these changes will boost accrual and allow us to complete the study. Furthermore, because the amended S9623 study calendar lists the four time points for S9719 blood specimen collection, no additional sample collection times are necessary. A copy of the protocol amendments may be found in the appendix (Exhibits 1 A and B).

Draft amendments for S9623/S9719 were submitted to the Department of Defense, Human Use Review Specialist, on June 15, 1999 for DOD review and approval. It was not anticipated that the formal written approval response from DOD and CTEP would take 13 months (written approval

received July 11, 2000). The amendments were incorporated into S9623/S9719 and were recently distributed in the Group mailings dated 7/15/00 and 9/15/00. The amendments are currently being re-evaluated by the IRBs for each Southwest Oncology Group member institution. Consequently, **the Southwest Oncology Group has not dispersed any DOD funds to support the research objectives outlined in this grant for Years 2 and 3.** Funding will be distributed to the testing centers when patient accrual resumes.

Controversy surrounding High Dose Chemotherapy in Breast Cancer. The use of high dose chemotherapy and autologous stem cell transplantation (HDC-ABMT) for the treatment of breast cancer remains a highly emotional and controversial issue. Preliminary results from several prospective randomized clinical trials examining the safety and efficacy of high dose chemotherapy with autologous stem cell rescue for the treatment of breast cancer have failed to show a survival advantage over conventional therapy; however, their results were hampered by limited power to detect moderate differences in survival due to small size constraints, short follow-up, and patient selection bias (1,2). Furthermore, the improved overall survival for HDC-ABMT reported in the South African study has been discounted by scientific irregularities and misconduct (3). Despite the lively discussions surrounding HDC-ABMT in breast cancer, the NCI views the treatment rationale of HDC-ABMT as sound. Resolution of this disagreement among the Medical Community will only be determined by patient participation in a large, carefully monitored clinical trial. To this end, Dr. Richard D. Klausner, Director, NCI, wrote a letter to the Medical Oncology Community urging their support of the high priority NCI-sponsored clinical trial, S9623 (clinical trial to S9719) to bring this question to closure (Exhibits 2 and 3). A May 2000 public opinion survey of 924 women ages 35-74 indicated that HDC-ABMT is of value for the treatment of breast cancer and the women firmly support the continuance of the NCI sponsored clinical trial (Exhibit 4). Despite the controversy and based on these recent endorsements, we remain optimistic that this DOD-funded biological investigation will eventually meet its target accrual of 200 patients. However, an extension is necessary to complete this study. The timelines described in the "original" Statement of Work could not have predicted the current controversy and the two years needed to seek approval from the Human Use Review Specialists to incorporate the biological DOD funded study into S9623 clinical protocol. We request permission to continue this study adjusting the timelines to be consistent with current accrual trend of the clinical protocol.

Experimental Design of S9719

Purpose: To test the hypothesis that genetic damage defined by the presence of clonal hematopoiesis can be detected in a subset of patients following dose-intensive adjuvant therapy.

Rationale for Clonal Hematopoiesis Project (S9719): Adjuvant therapy with anthracycline-based combination chemotherapy for patients with breast cancer has been shown to improve disease-free and overall survival. Unfortunately, therapy-related myelodysplasia (t-MDS) or therapy-related acute myeloid leukemia (t-AML) has emerged as uncommon, but well-established, complications of adjuvant therapy using dose-intensive regimens for breast cancer. According to the Jacobs' model of leukemogenesis (4), t-MDS or t-AML evolves as a result of expansion of an abnormal clone of hematopoietic stem cells, which have acquired somatic mutations conferring a growth advantage (Figure 1). This damage may result in clonal proliferation which, according to the Jacobs model of neoplasia, is an essential early (? initial) step in leukemogenesis, occurring prior to the development of clinical abnormalities.

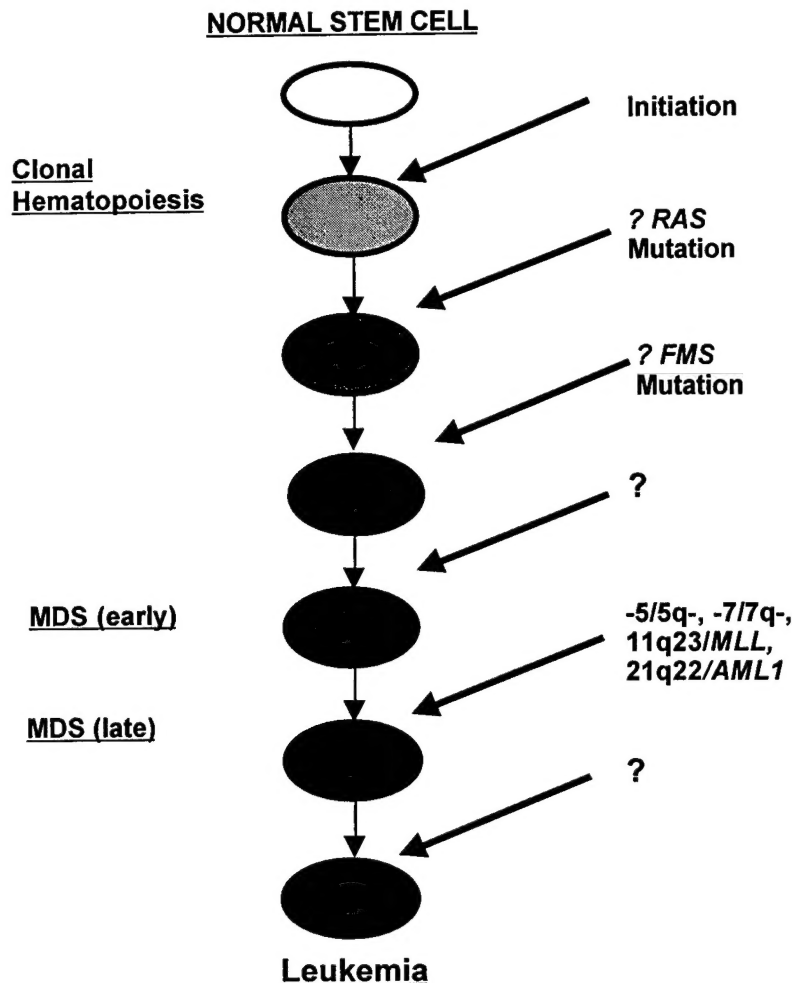


Figure 1. Jacobs' Model of Leukemogenesis (adapted from reference 4). According to the Jacobs model, t-MDS/AML evolves as a result of expansion of an abnormal clone of hematopoietic stem cells, which have acquired somatic mutations conferring a growth advantage. This damage may result in clonal proliferation which, according to the Jacobs model, is an essential early (? initial) step in leukemogenesis, occurring prior to the development of clinical abnormalities.

Study Population: Sequential blood (bone marrow when available) samples from 200 women enrolled in a single, randomized dose-intensive Southwest Oncology Group adjuvant breast cancer study for women with four or more positive nodes (S9623, "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with 4 or more Involved Axillary Lymph Nodes, Phase III, Intergroup").

Two ml of a pretreatment bone marrow aspirate and/or 40 ml of peripheral blood from each patient is collected in EDTA tubes. This sample serves as a sensitive control to detect any pre-treatment clonality abnormality. Forty ml of blood is also collected at three and twelve months following completion of all chemotherapy. For the 100 women in our study randomized to the autologous stem

cell transplant arm of S9623, 2 ml from the stem cell collection is also obtained for analysis. Samples obtained in years 1-3 were sent at room temperature by overnight courier to the Southwest Oncology Group tissue repository at the University of New Mexico, directed by Cheryl L. Willman, M.D, where specimens are separated by Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, New Jersey) into their mononuclear and granulocytic layers and frozen separately in liquid nitrogen. Samples were subsequently batched and sent to the City of Hope National Medical Center for nucleic acid isolation. Due to the low cell recovery after treatment, it was revealed that the entire sample must be used for the assays described in the protocol. After approval of this procedure modification, all future samples will be sent to the City of Hope National Medical Center for processing. A frozen cell bank will not be retained for samples submitted to this protocol.

Sample collection: Peripheral blood samples are collected from each patient enrolled on study at the time points listed in Table 1.

TABLE 1. Time points for Sample Collection for Study of Clonal Hematopoiesis
(Arm A – intensive chemotherapy; Arm B – autotransplant)

Time points		Time points	Sample Source
A.	Pretreatment	Arms A + B (200 Women)	Bone Marrow and/or Blood
B.	Stem Cell Collection	Arm B only (100 Women)	Apheresis (peripheral blood stem cells) <u>or</u> Bone Marrow Blood
C.	3 Months Following Completion of <u>All</u> Chemotherapy	Arms A + B (200 Women)	Blood
D.	12 Months Following Completion of <u>All</u> Chemotherapy	Arms A + B (200 Women)	Blood
E.	In case of diagnosis of Secondary Malignancy	Arms A + B	Bone Marrow Blood

*If the patient is also registered to S9702, pretreatment and stem cell collection samples may be collected for that study at the same time.

Despite the accrual concerns listed above, 22 patients have been registered to S9719 (clonal hematopoiesis protocol) from 13 Southwest Oncology Group centers. These institutions include Columbia River CCOP (n=7), Oregon Health Sciences University (n=2), St Francis/ Stormont/Kansas, U of (n=2), University of Arizona (n=1), Henry Ford Hospital (n=1), N Colorado Medical Center (n=1), Northwest CCOP (n=2), Salem Hospital (n=1), Loyola University (n=1), MD Anderson-FtWorth (n=1), St. Lukes/Mt States (n=1), Carilion Medical Center (n=1), and LSU-Shreveport (n=1).

As of 9/1/00, 123 samples from 21 patients have been processed. The sample submitted for the patient registered in July 2000 will be processed in the next batch. Three patients opted to come off study due to toxicity (n=1), extreme difficulty in collecting blood (n=1), or the patient became uncomfortable with proposed stem cell collection procedure (n=1).

Sample Processing: In year 3, frozen aliquots of mononuclear and granulocytes from all samples were sent by overnight service to Dr. Slovak's Laboratory nuclei acid isolation. Samples were

subsequently thawed and washed using previously described methods. An aliquot of 5×10^6 cells was frozen for RNA studies using RNA STAT 60 (Tel-Test, Inc., Friendswood, TX). T-cell separation was performed with antibody labeled magnetic Dynabeads M-450, CD2 (DynaL, Inc. Lake Success, NY). Granulocyte contamination is less than 1%, which has been verified by flow cytometry. High molecular weight DNA was prepared from the following standard proteinase K digestion and phenol/chloroform extraction methods (5, 6). The DNA is split equally for HUMARA (Dr. Marilyn Slovak) and microsatellite instability (Dr. Wendy Stock, University of Chicago) testing. Sample aliquots are shipped to Dr. Stock for RNA isolation (6). *MLL* RT-PCR testing and *RAS* mutation studies will not be performed until funds are distributed to the testing laboratory.

Assays Performed: Two different assays are used to detect clonality: 1) **HUMARA** (human androgen receptor) assay to estimate the incidence of early genetic damage defined by the presence of clonal hematopoiesis and 2) **Microsatellite instability (MSI)** testing to screen for loss of heterozygosity or the presence of defective DNA mismatch repair mechanisms.

HUMARA assay: The HUMARA assay is a PCR-based test to detect clonality utilizing the human androgen receptor locus on the X chromosome. The assay is highly informative due to a highly polymorphic CAG repeat (over 20 different alleles) in the first exon of the locus and the ability to quantitate allelic ratios between the active and inactive X chromosome (7, 8). Our protocol is a non-radioactive variation of the assay described by Mach-Pascual *et al.* (9) and quantitated by the method of Delabesse *et al.* (10). This assay amplifies a ~250 to 300 base pair (bp) region of the first exon of the human androgen receptor. Two *Hpa II* methylation sensitive sites reside within 100 bp 5' to the polymorphic CAG repeat (11) (Figure 2). Primers flank the methylation sensitive restriction enzyme sites and the CAG repeat simultaneously. Methylated enzyme (*Hpa II*) sites correlate with X inactivation. Unmethylated alleles (active X) are digested by *Hpa II* and eliminated from PCR amplification. The methylated or inactive allele will remain intact after the *Hpa II* digestion and is the only allele amplified. After amplification, the maternal and paternal alleles are resolved using a sequencing gel. Random inactivation will show both maternal and paternal alleles, signifying a polyclonal state; a clonal population will be identified by the presence of one allele or a shift of greater than 3-fold, to control for skewed X-inactivation over the other allele. The 5' primer is labeled with fluoroscein, with quantitation of alleles performed using Fluor-imager and ImageQuant software. Samples are run in duplicate and the entire assay is repeated twice to ensure reproducibility. Differences between mat and pat alleles, ranging between 3 to 40 bp.

Each sample comprises eight lanes; four lanes of T cell (control) DNA followed by four lanes of polymorphonuclear (test) DNA. *Rsa I*, a restriction enzyme that is not sensitive to DNA methylation status, cuts the DNA outside the trinucleotide repeat of the human androgen receptor gene locus, allowing for visualization of both alleles based on their differences in CAG repeat number. The first two lanes of control and test DNA are digested with *Rsa I* only to detect both maternal and paternal alleles and lanes 3 and 4 are digested with both *Rsa I* and *Hpa II* which determines inactive allele status only (Figure 3). Two correction factors are necessary for this assay; one factor corrects for preferential amplification of one of the two alleles and the second correction controls for skewed X-inactivation or excessive Lyonization. Those samples with a corrected ratio of less than 3 are considered within normal limits. Those with a corrected ratio greater than 3 are consistent with either skewed X inactivation or clonal hematopoiesis.

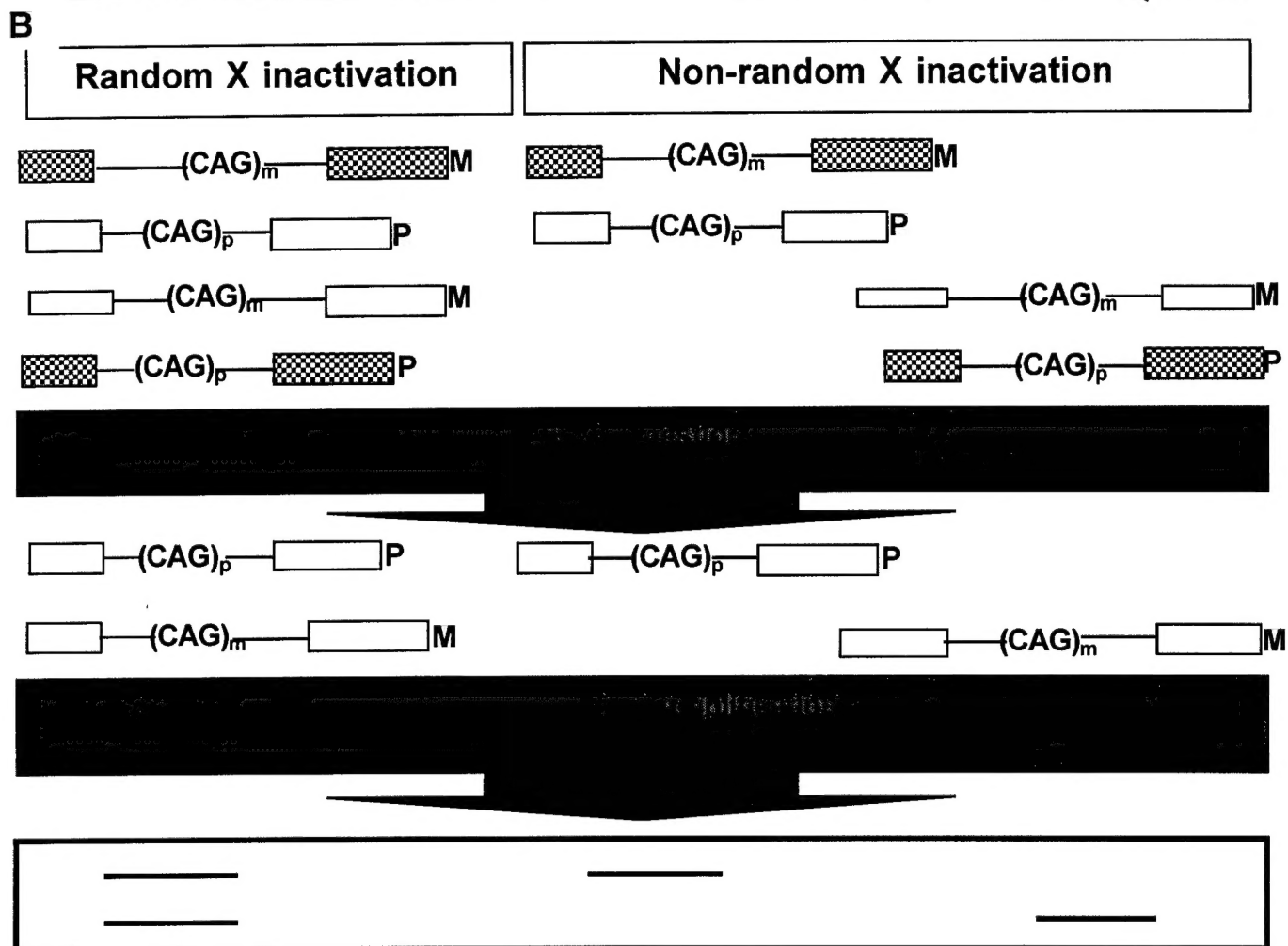


Figure 2. Humara Assay Schematic. (A) The assay amplifies a ~250 to 300 base pair (bp) region of the first exon of the human androgen receptor. Two *Hpa II* methylation sensitive sites reside within 100 bp 5' to the polymorphic CAG repeat. Primers flank the methylation sensitive restriction enzyme sites and the CAG repeat simultaneously (arrows). Methylated enzyme (*Hpa II*) sites correlate with X inactivation. (B) DNA is digested with *Rsa I* and *Hpa II* (Roche Diagnostic, Indianapolis, IN) restriction enzymes. Unmethylated alleles (active X) are digested by *Hpa II* and eliminated from PCR amplification. The methylated or inactive allele remains intact after the *Hpa II* digestion and is the only allele amplified. After amplification, maternal and paternal alleles are resolved using a sequencing gel. Random inactivation will show both maternal and paternal allele, signifying a polyclonal state; the presence of only one allele or shift of greater than 3-fold (to control for skewed X-inactivation over the other allele), will identify a clonal population. (Modified from reference 11)

Update of HUMARA results (September 1, 2000). Twenty of 21 patients have been informative by the HUMARA; one sample was monoallelic. Follow-up samples for 15 patients were submitted. All follow-up samples are within normal limits. For example, figure 3 shows sequential DNA samples submitted from breast cancer patient #167145, who received a peripheral blood stem cell transplant in April 1999. The peripheral blood and bone marrow samples collected at disease presentation exhibit a corrected ratio of 1.1 and 1.2 respectively. The apheresis sample collected after induction chemotherapy and at the time of stem collection has a corrected ratio of 1.0 whereas the sample collected on 10/21/99, at the 3-months post the completion of all chemotherapy, exhibited a corrected ratio of 1.3. All samples show polyclonal hematopoiesis with a corrected ratio of range of 1.0 to 1.3 over the seven-month time frame.

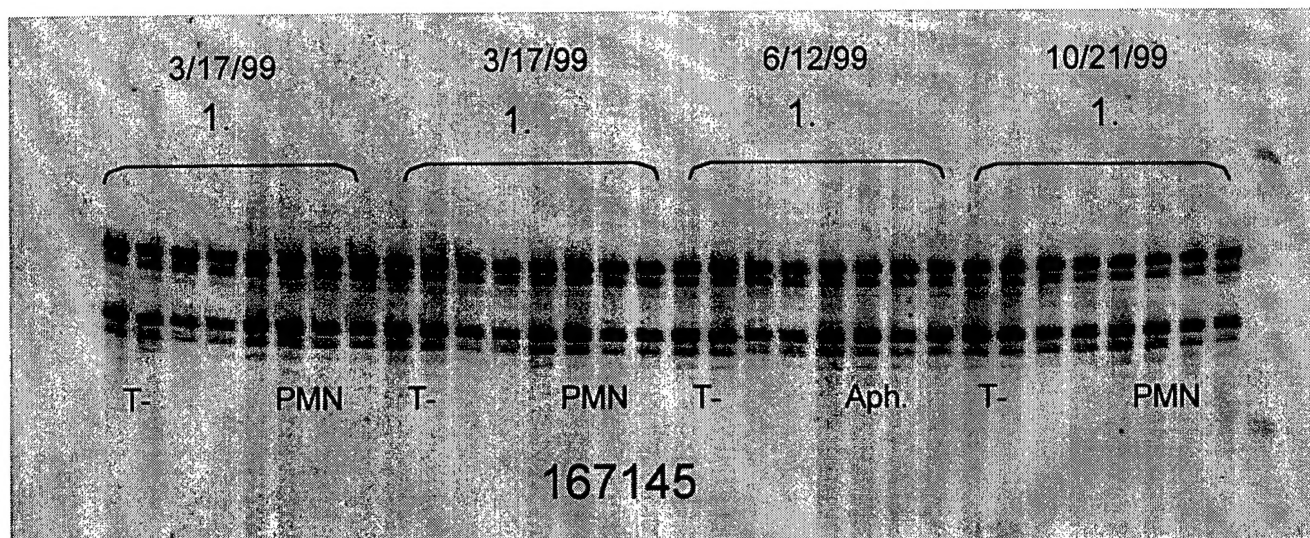


Figure 3. Sequential samples from patient #167145. This gel illustrates four samples from one S9719 patient: peripheral blood (lanes 1-8) and bone marrow (lanes 9-16) collected at disease presentation, the apheresis sample (lanes 17-24) collected 3 months later, and a blood sample collected 3 months after the completion of all therapy (25-32). Each sample comprises eight lanes; four lanes of T cell (control) DNA followed by four lanes of polymorphonuclear (test) DNA. The first two lanes of control or test DNA were digested with *Rsa I* and lanes 3 and 4 were digested with both *Rsa I* and *Hpa II*. Lanes 1,2,5,6,9,10,13,14,17,18,21,22,25,26,29 and 30 are *RSA I* digestion only; lanes 3,4,7,8,11,12,15,16,19,20,23,24,27,28,31 and 32 are digested with *Rsa I* and *Hpa II*. The patient demonstrates polyclonal hematopoiesis with a ratio range of 1.0 to 1.3 over the seven-month treatment period.

Excessive Lyonization refers to a skewed X-inactivation that occurs in females who have randomly inactivated a preponderance of one X-chromosome (either paternal or maternal X). Gale and colleagues (12) have estimated this occurs in ~23% of females and has been reported to increase with age, with >30% of the normal population having skewed XIP at age 60 (13). Because X inactivation patterns may vary from tissue to tissue, somatic controls from embryological related tissues are needed to determine and interpret skewed X inactivation patterns (14). The use of T cells, as the control tissue, eliminates false positives due to skewed X-inactivation that mimic a clonal population and controls for age related skewing. Previous studies that indicated that clonality studies using X-inactivation based assay should incorporate age-matched controls did not collect T-cells or perform corrected ratio to eliminate false positive due to skewed X-inactivation. In our study, the ratio of the two alleles in the experimental tissue (polymorphonuclear cells) must be

divided by the ratio of the same two alleles in normal somatic control tissue (T-cells). As above, if the ratio remains greater than 3, the results are consistent with clonal hematopoiesis.

Although our test population is very limited, corrected ratios using T cells from patients as internal control tissue has proven to be an excellent way to control for age-related skewing or excessive Lyonization in our test population. 20/21 patients tested to date have normal corrected clonal hematopoiesis ratios [exception being the monoallelic (non-informative) patient]. In another **prospective** study conducted at the City of Hope, T-cell corrected ratios appeared to provide adequate correction for skewed X-inactivation in patients (n=117) being treated with high dose chemotherapy with or without stem cell rescue for lymphoma, leukemia and multiple myeloma. Previously, we encountered problems associated with skewed inactivation due to our inability to obtain pretreatment (baseline) samples or the failure to obtain T-cells that serve tissue-specific internal controls for the patients. Accordingly, the need for age-matched female controls appears to be very important in **retrospective** studies in which the control tissue is not available. In this prospective study, the patient's T-cells are adequate control tissue to correct for the excessive skewing variable.

Microsatellite Instability Assays

Microsatellite instability (MSI) assays have been chosen as an alternative method to the HUMARA for detection of a clonal hematopoietic stem cell population emerging as a result of chemotherapy-induced genetic damage. The HUMARA and microsatellite assays are performed at two different institutions (Dr. Slovak's laboratory at the City of Hope National Medical Center and Dr. Stock's laboratory at University of Chicago). These assays are performed in a blinded fashion to obtain the highest degree of confidence. We have identified 10 MSI markers and have developed conditions for amplification and detection of these markers in tumor (granulocytic fraction of blood or mononuclear fraction of bone marrow) and control (peripheral blood T cell) populations (15-19).

The first 5 MSI markers were chosen on the basis of existing literature, documenting their utility in the detection of MSI in a variety of different malignancies. These markers include BAT26, BAT40, APC, Mfd15CA, and D2S123. The other five markers are also highly informative and were chosen on the basis of their location in genomic regions where chromosome translocations or loss of heterozygosity (LOH) have been frequently reported in therapy-related leukemias and, specifically, where abnormalities have been associated with topoisomerase II inhibitors. These MSI markers (with genomic location) include AFM240YA11 (3q21), AFM302xb9 (11q23), AFM031xc5 (21q22), AFM337zg5 (12p12) and AFMb298yh5 (20pter-20qter).

PCR primer pairs: Microsatellite marker primers, chromosome location and PCR conditions were described in the previous progress report. The forward primer of each primer set contains a 5'-end labeled fluorophore to allow for automated fluorescence detection. One fluorophore (6Fam, Hex, or Tet: blue, yellow, green fluorescence respectively) is end labeled to each forward primer.

PCR reactions: All PCR reactions contain 100 ng of either normal (T cell) or experimental (PMN) DNA and 2.5 units of Amplitaq Gold polymerase (Perkin-Elmer). The PCR reaction is a "hot start" reaction: The Amplitaq Gold polymerase must be heated initially at 95 °C for 12 minutes in order to be activated for amplification.

Parameters: 95°C x 12 min

95°C x 30 sec	}	45 cycles
x 30 sec		
72°C x 30 sec		
72°C x 10 min		
25°C hold		

Analysis: 2µl of each PCR product is run on a 4% polyacrylamide denaturing gel:

Each product is combined with a 350-bp size standard (Perkin-Elmer) labeled with Tamra (red fluorescent fluorophore).

The gels are run on an ABI 377 instrument and are analyzed following electrophoresis using Genescan Analysis software. The Genescan software collects raw signals emitted by each fluorophore. Every fragment in a peak contributes a single fluorophore: peak area is directly proportional to the number of molecules. The Genotyper DNA Fragment Analysis Software aids in determining the allele sizes of the amplified products. Therefore, the control and "tumor" allele sizes can be compared for determination of microsatellite instability.

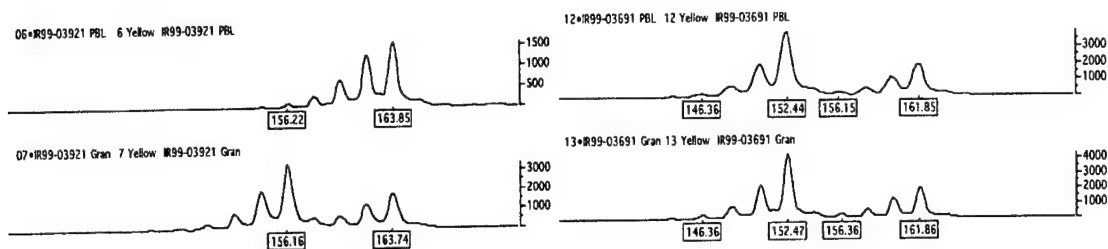
Results of Microsatellite Instability Assays (September 1, 2000). Of the 21 patients sampled, 20 patients showed no evidence of microsatellite instability, however, **one patient did show evidence of microsatellite instability** (figure 4) **prior to the initiation of any chemotherapy.** The patient's HUMARA ratio was 1.3, well within normal limits. The significance of this finding is not known. In the future, association of other pre-study patient characteristics and tumor-related variables, with presence or absence of clonality by HUMARA or microsatellite assays will also be explored.

Detection of *RAS* mutations and *MLL* gene rearrangements: In cases where the HUMARA or microsatellite repeat assays are positive for clonal hematopoiesis, sensitive reverse-transcriptase PCR assays will be used to determine whether *RAS* mutations and/or *MLL* fusion transcripts commonly reported in therapy-related myelodysplasia and AML have occurred. The methods for these assays have been previously reported (20,21). Currently, only one patient requires these additional studies. These studies will be performed when funds are available to perform the analyses and Dr. Stock, who has recently moved from the University of Illinois to the University of Chicago, receives IRB approval from the University of Chicago to perform the assays.

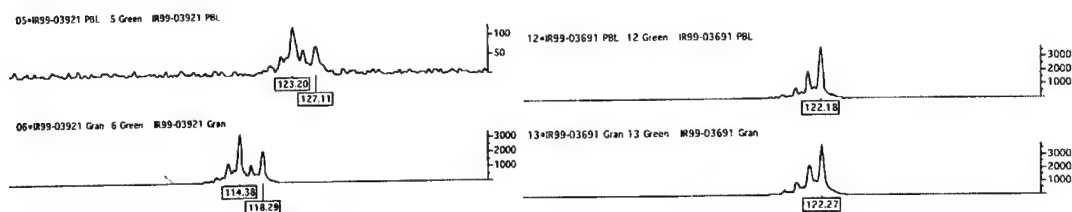
Panel A

Panel B

Mfd15



APC



D2S123

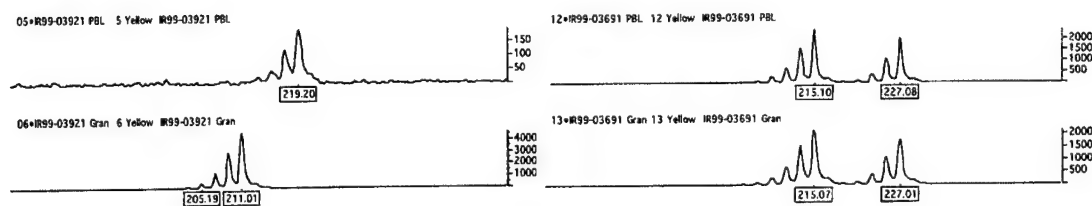


Figure 4. Testing for microsatellite instability (MSI) as a marker of clonal hematopoiesis. In the panels above, three loci (mfD15, APC, D2S123) are examined for the presence of MSI in the myeloid lineage of two patients with breast cancer. Samples were obtained prior to initiation of chemotherapy. Peripheral blood T lymphocytes (PBL) serve as the control tissue. Of each paired locus, the top electropherogram represent the PBL; the myeloid cells are shown in the lower electropherogram of each locus. In panel A, MSI is demonstrated in 3/3 loci. In comparison, panel B shows no MSI in the myeloid lineage.

KEY RESEARCH ACCOMPLISHMENTS

- Southwest Oncology Group biologic protocol (S9719) activated. Clonality assays developed. One hundred twenty-three samples from 21 patients analyzed to date.
- No evidence of clonal hematopoiesis by HUMARA assay detected. One patient was monoallelic (non-informative).
- One patient exhibited microsatellite instability prior to the initiation of therapy (pretreatment sample).
- The HUMARA and microsatellite instability assays give reproducible and complementary results using sequentially obtained blood samples of women treated on this study.
- Peripheral blood T-lymphocytes are a useful internal, tissue-specific control for the HUMARA assay precluding the need of age-matched controls for skewed X-inactivation.
- Protocol amendments to increase patient accrual were approved in year 2000 by the DOD and CTEP. Amendments (7/15/00 and 9/15/00) have been mailed to the Southwest Oncology Group institutions for institutional IRB approval.
- Despite the controversy among medical professionals, the NCI and public opinion survey support the continuance of the high priority, high dose chemotherapy with or without stem cell rescue clinical trial (S9623) for breast cancer patients.
- This DOD biological investigation (S9719) has been incorporated into the high priority NCI-sponsored clinical protocol (S9623) for Southwest Oncology Group institutions, lending support for the successful completion of the clonal hematopoiesis investigation.
- Analysis of additional patients with longer follow-up is essential to confirm these preliminary results; however, at this point, neither regimen used in this setting (dose-intensive therapy with growth factor support vs. high-dose therapy with stem cell reinfusion for stage I/II breast cancer) appears to **initiate** genetic damage that could result in development of hematologic malignancies.

REPORTABLE OUTCOMES

The data collected as of June 1, 2000 was presented at the Era of Hope 2000 meeting held in Atlanta, GA. Two abstracts (scientific and layperson) were submitted for distribution at the meeting (Exhibits 5 A and B).

CONCLUSIONS AND FUTURE DIRECTIONS

This pilot study was designed to test the hypothesis that genetic damage, defined by the presence of clonal hematopoiesis, can be detected in a subset of patients following dose-intensive adjuvant therapy on a current Southwest Oncology Group trial for breast cancer. The salient points outlined in the grant application's "Statement of Work" for years 1-3 have been addressed, however, low accrual has resulted in a major set back and the inability to meet the original timelines proposed. During the past two years, the highly emotional controversy of high dose chemotherapy with stem cell rescue for breast cancer has made a substantial impact on patient accrual to both the clonal hematopoiesis biological study and the clinical treatment protocol (S9623). The NCI and public opinion support the option of HDC-ABMT in the setting of a large randomized, carefully monitored clinical trial. The clinical trial that this biological investigation is associated with has received high

priority status to settle the current debate of comparable or improved disease free survival and overall survival in high-risk breast cancer patients with HDC-ABMT vs conventional therapy (Exhibits 2-4). We are cautiously optimistic that the approved amendment changes will boost accrual and allow us to complete the study. The protocol is simple and consists of four blood draws at four defined time points for those patients agreeing to participate in the study. The recent studies reporting a dose dependent increased risk of acute leukemia in breast cancer patients treated with mitoxantrone based regimens (22-24) but not in a sequential high dose doxorubicin followed by cyclophosphamide (25), further support the hypothesis of a causal relationship of prior therapy that requires further investigation. The biological questions of why this late effect complication only occurs in a subset of breast cancer patients, what the synergistic effects of other drugs or radiotherapy are, does the period/sequence of drug administration matter, are patients predisposed, and are faulty DNA repair mechanism at play, remain problematic in the treatment of breast cancer. Therefore, this DOD funded project is just as relevant and significant today as it was four years ago when the investigation was submitted for funding.

Confirmation that adjuvant chemotherapy induces clonal hematopoiesis in a significant number of patients from this pilot study will provide a unique model to prospectively study the evolution of therapy-related leukemogenesis in patients being treated for breast cancer, and would be the focus of a subsequent grant proposal. The goals of a larger study would include the following: 1) to determine whether a relationship exists between detection of clonal hematopoiesis and subsequent evolution to t-MDS/AML; 2) to identify general mechanisms (e.g., faulty DNA repair and mutations in components of cell cycle checkpoints, which may predispose patients to genetic instability and leukemogenesis, following adjuvant therapy; 3) to determine the sequence of events (genomic instability, loss of heterozygosity, specific mutations/translocations, etc.) which participate in leukemogenesis; and 4) to determine whether specific adjuvant regimens place patients at an unacceptably high risk for the development of therapy-related hematologic malignancies. If the study is negative, high risk breast cancer patients can rest assured that they can receive high dose chemotherapy, without an increase risk of development of clonal hematopoiesis or subsequent evolution to a therapy-related hematopoietic disorder over the general population.

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APPENDIX

Attached are the following appendices.

- Exhibit 1: Approved amendment protocols for S9719 and S9623
 - (a) Group Mailing dated 7/15/00
 - (b) Group Mailing dated 9/15/00

- Exhibit 2: Dr. Klausner's letter to Oncologists asks for support of S9623 to determine the best treatment option for high-risk breast cancer patients.

- Exhibit 3: NCI gives S9623/S9719 High Priority Status

- Exhibit 4: Omnibus Study indicates women between 35-74 would consider high dose chemotherapy with stem cell rescue as a treatment option for breast cancer.

- Exhibit 5: DoD Era of Hope Abstracts
 - (a) Scientific Abstract
 - (b) Layperson Abstract



Southwest Oncology Group

A National Clinical Research Group

July 15, 2000

TO: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS AND PATHOLOGISTS

FROM: Tamra N. Oner/Protocol Coordinator

RE: **S9719**, "Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence, Ancillary to **S9623**." Study Coordinators: Drs. M. Slovak, C. Willman, W. Stock, and K. Albain.

AMENDMENT #3

Study Coordinator: Marilyn L. Slovak, M.D. Phone: 818/359-8111 ext 2348
E-mail: mslovak@smtplink.coh.org

IRB Review Requirements

☒ Full board review required

☐ Expedited review allowed

☐ No review required

AMENDMENT #3

The above-noted study is now a mandatory ancillary to the Southwest Oncology Group treatment protocol, **S9623**. This requirement will remain in effect until accrual on **S9719** is completed. Specific protocol changes are listed below:

1. The word "AFFILIATE" replaces "CGOP" on the face page as this is the new Southwest Oncology Group standard terminology.
2. A sentence has been added to the end of the background section, Section 2.0, stating that "Any changes to this protocol require local IRB and HSRRB approval."
3. Sections 3.1 and 6.1 have been amended to state that patients must be registered to **S9719** at the same time they are registered to **S9623**, within the same registration phone call.
4. The word "hematologic" has been inserted into Section 4.1, Table 1, timepoint E and in Sections 4.2e and 7.9 in front of the word "malignancy" to clarify that bone marrow and blood are requested in cases of secondary hematologic malignancies. Also, the prior "*" footnote referencing **S9702** has been removed from Section 4.1, Table 1. It is no longer applicable as the **S9702** protocol has been closed. The "****" footnote symbols referring to the previous second footnote have been replaced with "*".

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5. The address for shipping of samples in Section 4.3a has been changed from University of New Mexico Cancer Center to the City of Hope National Medical Center. This has been done to ensure more efficient routing of samples and should reduce study related costs. Note: Study kits may still be ordered from the University of Mexico as specified in Section 4.2.
6. A new paragraph has been added to Section 8.0, Ethical and Regulatory Considerations, titled "REPORTING OF SERIOUS AND UNEXPECTED ADVERSE EVENTS" which includes adverse event reporting instructions.
7. Consent for participation in this study has been incorporated into the S9623 Model Informed Consent and the previous S9719 Model Informed Consent has been removed from the protocol.
8. A new "secondary" Model Informed Consent, entitled, "In case of Diagnosis of Secondary Hematologic Malignancy, Consent Form and Information About S9719", has been inserted into the Master Forms Set as Section 10.1.
9. Section 11.0, Appendix, has been repaginated due to replacement of the original Model Informed Consent with the secondary consent.

Replacement pages are attached for the face page, and pages 5, 6, 8, 10, 11, 15 - 17 and 20. Please append this memo to the front of your copy of the protocol and insert the replacement pages. Also, please replace the original Model Informed Consent with the new secondary consent form.

This memorandum serves to inform the Southwest Oncology Group Statistical Center, and the NCI.

cc: Stephanie Green, Ph.D.
Danika Lew, M.A
Diana Lowry
Camille White
Vicki Green
Karin Rantala
Marjorie Godfrey
Louise Pascal - DOD



**Southwest
Oncology Group**

A National Clinical Research Group

July 15, 2000

TO: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS, SURGEONS, RADIATION ONCOLOGISTS AND PATHOLOGISTS; CALGB, ECOG, NCCTG AND MDACC

FROM: Tamra N. Oner, Protocol Coordinator

RE: **S9623**, "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with 4-9 Involved Axillary Lymph Nodes, Phase III, Intergroup." Study Coordinators: Drs. S. Bearman, W. Peters and L. Pierce.

AMENDMENT #10

Study Coordinator: Scott I. Bearman, M.D. Phone: 303/372-9000
E-mail: sbearman@entente.uhcolorado.edu

IRB Review Requirements

- ☒ Full board review required
- ☐ Expedited review allowed
- ☐ No review required

AMENDMENT #10

The above-noted study has been amended to require that all patients registered to this study by Southwest Oncology Group institutions also be registered concurrently to the ancillary study **S9719**, "Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer; Pilot Study to Evaluate Incidence", within the same registration phone call. This requirement will remain in effect until accrual on **S9719** is completed. This requirement does not apply to other cooperative group participants on the study.

The specific protocol changes are listed below:

1. A new eligibility requirement requiring concurrent registration to **S9719** at the time of patient registration to **S9623** has been added as a new Section 5.11. The rest of Section 5.0 has been renumbered accordingly.
2. A new question has been added to the Eligibility Checklist as item #18 asking, "For patients enrolled by Southwest Oncology Group institutions, will the patient be registered to the companion protocol **S9719** immediately following the **S9623** registration AND during the same registration phone call? (Note: Indicate 'N/A' if the patient is being enrolled via another Cooperative Group OR if the patient refuses consent for **S9719**. If the patient refuses consent for **S9719**, this must be documented on the initial **S9623** flow sheet.)"
3. A new bolded sentence has been added after the first paragraph of Section 13.1 stating that, "Southwest Oncology Group institutions must register patients to the companion protocol, **S9719**, at the same time as **S9623** registration, within the same registration phone call unless the patient refuses to give consent for **S9719**. (If the patient refuses to give consent for **S9719**, this refusal must be documented on the initial **S9623** flow sheet.)"

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4. A new Section 15.8 has been added to Special Instructions, Section 15.0, specifying that Southwest Oncology Group institutions must enroll patients on the mandatory ancillary study, S9719, at the time of S9623 registration, within the same registration phone call. If the patient refuses to give consent to S9719, the refusal must be documented on the initial S9623 flow sheet.
5. A new Section XIII has been added to the Model Informed Consent as signature block for providing consent for participation in S9719. Also, the specifics of the S9719 Model Informed Consent have been added to the S9623 Model Informed Consent as specified below:
 - a. Information explaining the ancillary study, S9719, has been added to Section I as new paragraphs #7 and #8. The information in these paragraphs includes the purpose of the study, a description of the samples to be submitted and the identity of the laboratories and investigators performing the testing. Paragraph #7 also includes the phrase, "Bone marrow will only be submitted for this study if a specimen is available as a result of the treatment protocol."
 - b. A new second paragraph has been added to Section IV for patients participating in S9719. It specifies that there may be other ways of determining genetic damage comparable to the methods used in S9719. It also states that patients have the option of not having the procedure done on their blood (and marrow) samples.
 - c. A second paragraph has been added to Section VI specifying that for patients registered to S9719, there is no specific compensation provided for participation in the study.
 - d. A second paragraph has been added to Section VII for patients participating in S9719, stating that their records may be made available to the National Cancer Institute, the Food and Drug Administration, the Southwest Oncology Group and the U.S. Army Medical Research and Material Command.
 - e. Spaces for the patient's and witness's initials have been added to each page of the S9623 Model Informed Consent where there is no signature line.
6. A correction has been made in Section 5.8 and in Question #7 of the Eligibility Checklist under exclusion criteria. The phrase "both sides have < 10 nodes involved, one or more have 4 - 9 nodes involved" has been removed and replaced with "one or both sides have ≥ 4 nodes involved". This change was inadvertently left out of prior Amendment #9 dated March 1, 2000.

Replacement pages are attached for the Eligibility Checklist, pages 28, 50, 56b and 65 - 77a. Page 66a and 77a were added to prevent extensive repagination. Please append this notice to the front of your copy of the protocol and insert the replacement pages.

This memorandum serves to inform the Southwest Oncology Group Statistical Center, ECOG, CALGB, NCCTG, MDACC and the NCI.

cc: Stephanie J. Green, Ph.D.
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**Southwest
Oncology Group**

A National Clinical Research Group

July 15, 2000

TO: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS AND PATHOLOGISTS

FROM: Tamra N. Oner/Protocol Coordinator

RE: **S9719**, "Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence, Ancillary to **S9623**." Study Coordinators: Drs. M. Slovak, C. Willman, W. Stock, and K. Albain.

AMENDMENT #3

Study Coordinator: Marilyn L. Slovak, M.D. Phone: 818/359-8111 ext 2348
E-mail: mslovak@smtplink.coh.org

IRB Review Requirements

(☒) Full board review required

(☐) Expedited review allowed

(☐) No review required

AMENDMENT #3

The above-noted study is now a mandatory ancillary to the Southwest Oncology Group treatment protocol, **S9623**. This requirement will remain in effect until accrual on **S9719** is completed. Specific protocol changes are listed below:

1. The word "AFFILIATE" replaces "CGOP" on the face page as this is the new Southwest Oncology Group standard terminology.
2. A sentence has been added to the end of the background section, Section 2.0, stating that "Any changes to this protocol require local IRB and HSRRB approval."
3. Sections 3.1 and 6.1 have been amended to state that patients must be registered to **S9719** at the same time they are registered to **S9623**, within the same registration phone call.
4. The word "hematologic" has been inserted into Section 4.1, Table 1, timepoint E and in Sections 4.2e and 7.9 in front of the word "malignancy" to clarify that bone marrow and blood are requested in cases of secondary hematologic malignancies. Also, the prior "***" footnote referencing **S9702** has been removed from Section 4.1, Table 1. It is no longer applicable as the **S9702** protocol has been closed. The "***" footnote symbols referring to the previous second footnote have been replaced with "".

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9. Section 11.0, Appendix, has been repaginated due to replacement of the original Model Informed Consent with the secondary consent.

Replacement pages are attached for the face page, and pages 5, 6, 8, 10, 11, 15 - 17 and 20. Please append this memo to the front of your copy of the protocol and insert the replacement pages. Also, please replace the original Model Informed Consent with the new secondary consent form.

This memorandum serves to inform the Southwest Oncology Group Statistical Center, and the NCI.

cc: Stephanie Green, Ph.D.
Danika Lew, M.A
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SOUTHWEST ONCOLOGY GROUP

**CLONAL HEMATOPOIESIS AS A MARKER OF GENETIC DAMAGE FOLLOWING ADJUVANT
CHEMOTHERAPY FOR BREAST CANCER: PILOT STUDY TO EVALUATE INCIDENCE**

ANCILLARY TO S9623

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PARTICIPANTS: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS AND PATHOLOGISTS	

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The following pages 26-42 are replacement pages to the original Southwest Oncology Group Protocol.

using pretreatment (baseline) samples to control for variables such as age or damage that may have occurred due to other risk factors/exposures. This study will only evaluate high risk patients (no low risk patients). Two different assays (the HUMARA and microsatellite instability) will be used to detect clonal hematopoiesis as a marker of genetic damage in this pilot study. **S9623** will compare the clinical outcome produced with autologous peripheral blood progenitor cell (PBPC) supported high-dose therapy with that of intensive, sequential chemotherapy in Stage II/III breast cancer patients involving 4 - 9 axillary lymph nodes.

PLEASE NOTE: Any changes to this protocol will require local IRB and HSRRB approval.

Inclusion of Women and Minorities:

This study involves only patients previously registered to **S9623**. No new patients (not enrolled on **S9623**) will be registered.

3.0 **ELIGIBILITY CRITERIA**

Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration. Use the spaces provided to confirm a patient's eligibility. **This section does not need to be submitted to the Statistical Center.**

- _____ 3.1 Patients with Stage II/III breast cancer involving 4 - 9 axillary lymph nodes enrolled on **S9623** will be eligible for this study. **Patients must be registered at the time of registration to S9623, within the same registration phone call.**
- _____ 3.2 A pretreatment sample of forty (40) ml of peripheral blood (four 10 ml EDTA tubes supplemented with 2 ml of tissue culture medium) or four provided tubes (per Section 4.2) and a 2 - 4 ml aliquot of pretreatment bone marrow aspirate, when available, (collected in EDTA tubes supplemented with tissue culture medium or provided tubes) must be collected prior to beginning treatment on **S9623**.
- _____ 3.3 All patients must be informed of the investigational nature of this study and must sign and give written informed consent in accordance with institutional and federal guidelines.
- _____ 3.4 At the time of patient registration, the date of institutional review board approval for this study must be provided to the Statistical Center.

4.0 **PROCEDURES/SAMPLE SUBMISSION REQUIREMENTS**

- 4.1 **Study Design.** For this pilot, samples will be obtained prior to initiation of treatment, from collected stem cell specimens (in 100 patients randomized to autologous stem cell transplant arm), and at three and twelve months following completion of treatment. For details of sample time points see Table 1 below.

TABLE 1. Time points for Sample Collection for Study of Clonal Hematopoiesis
(Arm A - intensive chemotherapy; Arm B- autotransplant)

Timepoints	S9623	Sample Source
A. Pretreatment*	Arms A + B	Bone Marrow (<u>when available</u>) Blood
B. Stem Cell Collection* (at ~ 3 months)	Arm B only	Apheresis specimen or Bone Marrow harvest specimen Blood
C. 3 Months Following Completion of <u>All</u> Chemotherapy*	Arms A +B	Blood
D. 12 Months Following Completion of <u>All</u> Chemotherapy*	Arms A + B	Blood
E. In case of diagnosis of Secondary Hematologic Malignancy	Arms A + B	Bone Marrow Blood

*These samples should be submitted even if the patient discontinues **S9623** treatment early (see Section 4.2c - 4.2d).

4.2 **Sample Collection:**

MAILING TUBES FOR SAMPLE SUBMISSION

Specially packaged kits with EDTA tubes (identical to those kits used for Southwest Oncology Group leukemia protocols) will be provided for this study. You may obtain these kits by calling

Sheryl L. Curtin, CMCD Research Facility Coordinator
University of New Mexico Cancer Center
2325 Camino de Salud NE, Room 101
Albuquerque, NM 87131
Telephone: 505/272-8881

Please call (preferably by 12 noon M.T.) the day before patient registration, in order that the kits will arrive to you by Federal Express for next day delivery. These tubes may be ordered ahead of time and stored frozen until use.

This kit contains specially provided EDTA tubes containing 2 ml STERILE tissue culture medium (RPMI-1640) supplemented with 10% fetal calf serum, 20 mg/ml Na₂ EDTA, pH 7.4. Replacement kits may be obtained by calling Sheryl L. Curtin.

- d. **Twelve months following completion of all chemotherapy.** Applies to all patients enrolled on this study. Samples will be collected from each patient at twelve months following completion of all chemotherapy on either arm of **S9623**. These samples should be submitted even if the patient discontinues **S9623** treatment early, twelve months after off-treatment.

Forty ml of **peripheral blood** (four 10 ml EDTA tubes supplemented with 2 ml tissue culture medium) must be collected from each patient.

- e. **Additional samples.** If there is diagnosis of a secondary hematologic malignancy including MDS or AML, a bone marrow aspirate and blood sample is requested.

1. Two ml of **bone marrow** aspirate from each patient collected into EDTA tubes supplemented with tissue culture medium.
2. Forty ml of **peripheral blood** (four 10 ml EDTA tubes supplemented with 2 ml tissue culture medium) must be collected from each patient.

4.3 **Handling of Required Study Samples.** All samples should be sent to Dr. Marilyn Slovak at room temperature by overnight courier to arrive within 24 hours at the City of Hope National Medical Center.

a. **Bone marrow and blood samples:**

All bone marrow, blood and apheresis samples will each be placed in EDTA tubes supplemented with tissue culture medium and sent **the day it is obtained** by Federal Express to:

City of Hope National Medical Center
1500 East Duarte Road
Northwest Building, Room 2265
Duarte, CA 91010
Phone: 626/359-8111 ext. 2025

Tubes should be labeled with the patient's name, Southwest Oncology Group patient number, study number (**S9719**), the date and time of collection, and type of product (marrow, apheresis, or peripheral blood). Each tube should be tightly capped and wrapped with paraffin film to prevent leakage. The tube should then be placed in a standard biohazard specimen resealable bag.

5.0 **STATISTICAL CONSIDERATIONS**

One hundred patients per arm from **S9623** will be accrued on this study. The length of accrual is anticipated to be 3 years. Compliance with the three month blood draw should be nearly complete; at 12 months following completion of treatment, approximately 15% might be anticipated to have relapsed or refused and not have samples available. The probability of clonal hematopoiesis at a particular time point can be established to within ± 0.1 with a sample size of 100 per arm, and to within ± 0.11 with a sample size of 85. Change in status between pretreatment, stem cell collection, and the three and twelve month post-treatment samples will be explored, as will concordance of the HUMARA and microsatellite assays. Association of treatment, pre-study, patient characteristics, and tumor-related variables with presence or absence of clonality by HUMARA or microsatellite assays will also be explored. For example, a two-sided .05 level test of the association of the treatment group with presence or absence of clonality will have adequate power to detect differences of .25 or greater (power at least .93 for the pretreatment and three month time point and .88 for the 12 month post-treatment time point, if sample size decreased to 85 per arm).

6.0 **REGISTRATION GUIDELINES**

- 6.1 Patients must be registered on this protocol **at the same time they are registered to S9623, within the same registration phone call.**
- 6.2 At the time of registration, the caller must be prepared to answer every question on the **S9719** Registration Form.
- 6.3 The caller must also be prepared to provide the date of institutional review board approval for this study. Patients will not be registered if the IRB approval date is not provided or is > 1 year prior to the date of registration.

7.0 **DATA SUBMISSION SCHEDULE**

- 7.1 Data must be submitted according to the protocol requirements for **ALL** patients registered, including patients deemed to be ineligible. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.
- 7.2 Master forms are included in Section 10.0 and (with the exception of the sample consent form) must be photocopied for data submission to the Southwest Oncology Group Statistical Center.
- 7.3 Members and CCOPs must submit one copy of all data forms directly to the Statistical Center in Seattle. CGOPs must submit (number of copies to be determined by the Group Member) copies of all forms to their Group Member institution for forwarding to the Statistical Center.
- 7.4 **AT REGISTRATION:**

Submit pre-study bone marrow (when available) and blood per Sections 4.2a and 4.3 to the University of New Mexico.

7.5 WITHIN FOURTEEN DAYS OF REGISTRATION:

Submit copies of the **S9719** Registration Form.

7.6 AT APHERESIS (FOR PATIENTS ON ARM B ONLY):

Submit apheresis blood samples (or bone marrow harvest if patient undergoes bone marrow harvest) and peripheral blood per Sections 4.2b and 4.3 to the University of New Mexico.

7.7 THREE MONTHS AFTER OFF-TREATMENT:

Submit blood per Sections 4.2c and 4.3 to the University of New Mexico.

7.8 TWELVE MONTHS AFTER OFF-TREATMENT:

Submit blood per Sections 4.2d and 4.3 to the University of New Mexico.

7.9 AT DIAGNOSIS OF SECONDARY HEMATOLOGIC MALIGNANCY:

Submit bone marrow and blood per Sections 4.2e and 4.3 to the City of Hope National Medical Center.

8.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent

The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Institutional Review

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

REPORTING OF SERIOUS AND UNEXPECTED ADVERSE EVENTS

Adverse experiences that are both serious and unexpected will be immediately reported by telephone to the USAMRMC Deputy Chief of Staff for Regulatory Compliance and Quality (301/619-2165) (non-duty hours call 301/619-2165 and send information by facsimile to 301/619-7803). A written report will follow the initial telephone call within 3 working days. Address the report to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RCQ, 504 Scott Street, Fort Detrick, MD 21702-5012.

10.0 MASTER FORMS SET

This section includes copies of all data forms which must be completed for this study. These include:

- 10.1 **S9719** Secondary Model Consent Form (replaced with "In Case of Diagnosis of Secondary Hematologic Malignancy, Consent Form and Information about **S9719**" as of 7/15/00).
- 10.2 **S9719** Registration Form
- 10.3 Specimen Submission Form

Amended 7/15/00

This model informed consent form has been reviewed by the DCT/NCI and is the official consent document for this study. Local IRB changes to this document are allowed. (Institutions should attempt to use sections of this document which are in bold type in their entirety.) Editorial changes to these sections may be made as long as they do not change information or intent. If the institutional IRB insists on making deletions or more substantive modifications to the risks or alternatives sections, they may be justified in writing by the investigator and approved by the IRB. Under these circumstances, the revised language, justification and a copy of the IRB minutes must be forwarded to the Southwest Group Operations Office for approval before a patient may be registered to this study.

**IN CASE OF DIAGNOSIS OF SECONDARY HEMATOLOGIC MALIGNANCY,
CONSENT FORM AND INFORMATION ABOUT S9719 , " CLONAL HEMATOPOIESIS AS A MARKER
OF GENETIC DAMAGE FOLLOWING ADJUVANT CHEMOTHERAPY FOR BREAST CANCER: PILOT
STUDY TO EVALUATE INCIDENCE" ANCILLARY TO S9623
TO BE CONDUCTED AT**

- I. **You had previously been registered to and taken part in this study at the time that you were treated for breast cancer on the Southwest Oncology Group study, S9623. At that time you had blood (and possibly bone marrow) samples submitted for genetic testing. You are now invited to submit additional blood and bone marrow samples because you have been diagnosed with a secondary hematologic malignancy. The purpose of this study (S9719) is to learn whether the treatment for your breast cancer may have caused gene damage to your hematopoietic cells (early blood cells) which may be associated with development of hematologic malignancies in a small subgroup of breast cancer patients.**

No additional bone marrow examinations (others than those required as part of your standard care for the diagnosis and treatment of your hematologic malignancy) will be required for this study. Small amounts of your blood and bone marrow will be sent to a central laboratory for testing.

We cannot and do not guarantee you will benefit if you take part in this study. If you take part in this study, the studies done on your blood cells may lead to discoveries which help future patients with breast cancer.

- II. **As part of your diagnosis and treatment for your hematologic malignancy, you will have cells collected from your bone marrow and peripheral blood. Part of the bone marrow and blood samples taken for your treatment will be submitted for this study. The submission of these samples is very important for this study and will help in finding out if genetic damage is related to a second cancer. By signing this form, you are agreeing to have these bone marrow and blood submitted for the purposes of this study.**

Patient initials _____

Witness initials _____

- III. **There may be other ways of determining if there is genetic damage in your cells. The methods used in this study are comparable to others that may be available. You also have the option of not having this procedure done on your blood and bone marrow samples.**
- IV. The process of bone marrow harvests and collection of peripheral blood should have already been explained to you by the doctor treating you for your breast cancer.
- V. We will keep any information we learn from this study confidential and disclose it only with your permission. By signing this form, however, you allow us to make your records available to the National Cancer Institute, the Food and Drug Administration, the U.S. Army Medical Research and Material Command and the Southwest Oncology Group. If we publish the information we learn from this study in a medical journal, you will not be identified by name. You may request a copy of the study results after the study is finished.
- VI. Whether or not you take part in this part of the study will not affect your future relations with your doctors (there will be no loss of benefit or change in attitude) or _____ (hospital name). If significant new findings are developed during the course of this study which may relate to your willingness to continue, this information will be provided to you. In addition, you understand that you may refuse to continue on this study, at any time after the start of therapy, without fear of prejudice to additional treatment that may be needed.
- VII. The doctor(s) involved with your care can answer any questions you may have about the drug program. In case of a problem or emergency, you can call the doctors listed below day or night.
- | | <u>Office</u> | <u>Home</u> |
|-----|---------------|-------------|
| Dr. | | |
| Dr. | | |
| Dr. | | |
- You can also call the Institutional Review Board(#_____) if you have any questions, comments or concerns about the study or your rights as a research subject.
- VIII. We will give you a copy of this form to keep.
- IX. You are deciding whether or not to take part in this part of the study. If you sign, it means that you have decided to volunteer after reading and understanding all the information on this form.

Date

Signature of Subject

Signature of Witness

Signature of Investigator

Printed or Typed Name of Witness

Time



Southwest Oncology Group

A National Clinical Research Group

August 1, 2000

TO: ALL SOUTHWEST ONCOLOGY GROUP, CCOP AND AFFILIATE MEDICAL ONCOLOGISTS, SURGEONS, RADIATION ONCOLOGISTS AND PATHOLOGISTS; CALGB, ECOG, NCCTG AND MDACC

FROM: Tamra N. Oner, Protocol Coordinator

RE: **S9623**, "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with ≥ 4 Involved Axillary Lymph Nodes, Phase III, Intergroup." Study Coordinators: Drs. S. Bearman, W. Peters and L. Pierce.

REVISION #7

Study Coordinator: Scott I. Bearman, M.D. Phone: 303/372-9000
E-mail: sbearman@entente.uhcolorado.edu

IRB Review Requirements

- () Full board review required
- () Expedited review allowed
- (√) No review required

REVISION #7

The requirement for Southwest Oncology Group institutions to offer the ancillary study, **S9719**, "Clonal Hematopoiesis as a Marker of Genetic Damage Following Adjuvant Chemotherapy for Breast Cancer: Pilot Study to Evaluate Incidence, Ancillary to **S9623**", to all patients registered to the above-noted study will become **effective September 15, 2000**. This is to allow time for institutions to conduct Institutional Review Board review and approval of the **S9719** protocol, if necessary.

Also, a pagination error has been corrected. Page 50 was incorrectly copied onto the back of page 49 with Amendment #10, dated July 15, 2000. Page 49a should have been copied onto the back of page 49, with page 50 as a single-sided page. Thus, pages 49, 49a and 50 are included with this revision although their content has not been revised.

The effective date has been noted in Section 5.11 and Question #18 of the Eligibility Checklist. Enclosed are replacement pages for pages 28, 49, 49a, 50 and the Eligibility Checklist. Please attach this memo to the front of your copy of the protocol and insert the replacement pages.

This memorandum serves to inform the Southwest Oncology Group Statistical Center, ECOG, CALGB, NCCTG, MDACC and the NCI.

cc: Stephanie J. Green, Ph.D.
Danika Lew, M.A.
Lauren Crowley
Diana Lowry
Karin Rantala
Jean Barce
Jean MacDonald - ECOG

Kathleen Karas - CALGB
Janis Gjervik - NCCTG
Debbie Frye - MDACC
Teresa Fratacangeli - MDACC
Brian Koziol - Amgen
Marjorie A. Godfrey

Operations Office

14980 Omicron Drive • San Antonio, TX 78245-3217 • Telephone 210-677-8808 • FAX 210-677-0006 • <http://www.oo.saci.org>



July 1, 1996

*SWOG Statistical Center
Fred Hutchinson Cancer Research Center
1124 Columbia Street MP557
Seattle, WA 98104-2092
Patient registration (206) 667-4623*

SWOG Patient No.	
Treatment No.	
Other Group Patient No.	
Other Group Name	

Investigator No.		<u>PATIENT NAME</u> (last,first,middle)	
Investigator		Patient's sex and race	
Institution		Patient's birthdate	
Date of IRB approval		Patient's Soc. Sec. No.	
Date of informed consent		Patient's zip code	
Projected start date of treatment		Method of payment	

S9623 "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with ≥ 4 Involved Axillary Lymph Nodes."

Eligibility Checklist

Each of the questions in the following two sections must be answered appropriately for a patient to be considered eligible for registration. The checklist should be entirely filled out and should be referred to during the phone registration. A copy must be submitted with the Prestudy form and initial flow sheet.

Criteria for Eligibility (All responses must be *Yes*)

Yes No

- _____ 1. Does the patient have histologically confirmed adenocarcinoma of the female breast with ≥ 4 histologically involved axillary and/or intramammary lymph nodes?
- _____ 2. Has the patient undergone a modified radical mastectomy or breast sparing surgery, plus an axillary node dissection?
- _____ 3. Are surgical margins negative for invasive and non-invasive ductal carcinoma?
- _____ 4. Is the interval between definitive surgery and the date of registration ≤ 12 weeks?
(Note: The date of definitive surgery is defined as the date of axillary dissection if the patient had only a breast sparing procedure; otherwise, it is the mastectomy date.)
Date of surgery _____
- _____ 5. Have a minimum of 10 nodes been sampled?



Eligibility Checklist

S9623

SWOG Patient No.	
Other Group Patient No.	

- ____ 6. Does the patient have a Southwest Oncology Group performance status of 0 or 1 per Section 10.4?
- ____ 7. Have the staging requirements per section 5.5 been completed within 60 days prior to registration and are the results negative for metastatic disease?
- Date of chest x-ray _____
- Date of bilateral bone marrow aspirates and biopsies _____
- Date of chest CT scan _____
- Date of abdominal/pelvic CT scan _____
- Date of head CT scan, if symptoms present _____
- (Note: If no symptoms of CNS involvement are present, indicate N/A.)
- Date of bone scan _____
- ____ 8. Does the patient have adequate organ function as defined by the following chemistries performed within 28 days prior to registration?
- SGOT $\leq 1.5 \times$ IULN? Value _____ IULN _____
- Bilirubin $\leq 1.5 \times$ IULN? Value _____ IULN _____
- WBC $\geq 3000/\mu\text{l}$? Value _____
- ANC $\geq 1000/\mu\text{l}$? Value _____
- Platelets $\geq 100,000/\mu\text{l}$? Value _____
- Calculated or 24-hour Creatinine Clearance ≥ 60 ml/minute?
- Value _____ Method (circle one): calculated / 24-hour
- Date obtained (oldest if chems obtained on different dates) _____
- ____ 9. Does the patient have pulmonary function tests showing FVC, FEV1, and DLCO all $\geq 60\%$ of predicted performed within 84 days prior to registration?
- FVC (%) _____ FEV1 (%) _____ DLCO (%) _____
- Date obtained (oldest) _____
- ____ 10. Has the patient had a normal ECG performed within 84 days prior to registration; or, in the event of an abnormal ECG, has the patient been cleared by a cardiologist?
- Date obtained _____
- ____ 11. Has the patient had a resting MUGA with the LVEF $\geq 45\%$ performed within 84 days prior to registration?
- LVEF (%) _____
- Date obtained _____
- ____ 12. If the patient is on medication known to alter cardiac conduction, has she been cleared by a cardiologist? (Note: Indicate N/A if patient is not on medication affecting cardiac conduction.)



Eligibility Checklist

S9623

SWOG Patient No.	
Other Group Patient No.	

- _____ 13. Is the patient HIV negative and has testing been performed within 42 days prior to registration?
Date of HIV test _____
- _____ 14. Are hepatitis B surface antigen and hepatitis C status known, and has testing been performed within 42 days prior to registration?
Date of hepatitis B and C tests _____
- _____ 15. Has a mammogram of the opposite breast been performed within 16 weeks prior to registration, and were the results one of the following: 1) normal, 2) abnormal but abnormalities determined not to be breast cancer, or 3) synchronous breast cancer?
Date of opposite breast mammogram _____
- Indicate 'N/A' if this patient has undergone bilateral mastectomies.**
- _____ 16. Has the patient been evaluated and approved for S9623 by a transplant center approved for this study by one of the participating cooperative groups per section 19.4 prior to registration? (Note: This evaluation includes confirmation of bed availability at the transplant center and confirmation that a written request for insurance approval has been submitted [and/or confirmation of ability to pay for transplant].)
Name of approved transplant center _____
- _____ 17. If the patient is of reproductive potential, has she agreed to use effective contraception while on this protocol? (Note: Indicate N/A if not of reproductive potential.)
- _____ 18. **Effective 9/15/00:** Will the patient be registered to the companion protocol, S9719, immediately following the S9623 registration AND during the same registration phone call? Indicate 'N/A' if the patient is being registered by a non-Southwest Oncology Group institution. Indicate 'N/A' if the patient is being registered prior to 9/15/00. For Southwest Oncology Group institutions: Indicate 'N/A' if the patient refuses consent for S9719 – this is the only exception allowed and must be documented on the initial S9623 flow sheet.

Criteria for Exclusion (All responses must be *No*)

Yes No

- _____ 1. Does the patient have known N3 disease, or T4 or M1 disease?
- _____ 2. Has the patient received prior chemotherapy for any malignancy?
- _____ 3. Has the patient received prior radiotherapy to the breast or prior hormonal therapy for breast cancer?
- _____ 4. If any additional studies were performed at the discretion of the investigator per section 5.5, were they reported as positive for malignancy?
(Note: If no additional studies were done, indicate N/A.)
- _____ 5. Does the patient have any congestive heart failure, serious cardiac conduction abnormalities such as second or third degree heart block, atrial or ventricular arrhythmias, or other uncontrolled or significant cardiovascular disease?
- _____ 6. Has the patient had a prior malignancy? (Exceptions: Adequately treated basal cell or squamous cell skin cancer, *in situ* cervical cancer, intraductal or lobular carcinoma *in situ* of the breast [diagnosed at any time], or any other cancer from which the patient has been disease-free for five years.)



Eligibility Checklist

S9623

SWOG Patient No.	
Other Group Patient No.	

- _____ 7. Does the patient have bilateral breast cancer? (Exceptions: Synchronous [diagnosed at the same time, i.e. within 4 weeks of initial histologic diagnosis] carcinoma of the contralateral breast, provided neither tumor is N3 or T4, both sides have <10 nodes involved, one or both sides have 4-9 nodes involved, and either a modified radical mastectomy or breast sparing surgery plus an axillary dissection [meeting the criteria in Section 5.2] has been performed for both tumors.)
- _____ 8. Does the patient have any serious medical or psychiatric illness which prevents informed consent or participation in this trial?
- _____ 9. Is the patient pregnant or nursing?

Stratification Factors (Response does not affect eligibility)

1. Primary Treatment
_____ mastectomy and no RT
_____ mastectomy and RT following chemotherapy
_____ breast sparing procedure and RT following chemotherapy

(Note: The decision regarding post-mastectomy radiation therapy must be made prior to randomization. A change in plan after randomization will constitute a major protocol violation.)

Descriptive Factors

1. Menopausal Status
_____ premenopausal
_____ postmenopausal
_____ other
2. Receptor Status (indicate [+], [-], or unknown)
ER _____
PgR _____
3. N2
Yes _____
No _____
4. T3
Yes _____
No _____
5. Transplant Regimen
Stamp I _____
Stamp V _____
6. Source of Progenitor Cells
Marrow _____
Peripheral Blood _____
Both _____

- b. CBC (WBC \geq 3,000/ μ l, ANC \geq 1,000/ μ l, platelets \geq 100,000/ μ l) within 28 days prior to registration.
 - c. 24 hour urine for creatine clearance or calculated creatinine clearance by the formula below (must be \geq 60 ml/minute) within 28 days prior to registration.
Estimated creatinine clearance =
$$\frac{(140 - \text{age}) \times \text{wt (kg)} \times 0.85}{72 \times \text{creatinine (mg/dl)}}$$
 - d. Pulmonary function tests showing FVC, FEV1, DLCO all \geq 60% of predicted within 84 days prior to registration.
 - e. ECG within 84 days prior to registration. In the event of abnormal ECG, candidates must be cleared by a cardiologist.
 - f. Resting MUGA with left ventricular ejection fraction \geq 45% within 84 days prior to registration.
 - g. No uncontrolled or significant cardiovascular disease; no congestive heart failure; no serious cardiac conduction abnormalities such as second or third degree heart block; no atrial or ventricular arrhythmias. Patients who are on medication known to affect cardiac conduction are eligible if they receive these medications for reasons other than heart failure or arrhythmia and are cleared by a cardiologist.
 - h. Patients must be HIV negative. Hepatitis B surface antigen and hepatitis C status must be known. Hepatitis and HIV testing must be performed within 42 days prior to registration.
- 5.7 Mammogram of the opposite breast must be performed within 16 weeks of registration. Results must be one of the following: normal, abnormal but abnormalities determined not to be breast cancer, or synchronous breast cancer. Patients who have undergone bilateral mastectomies are not required to undergo mammography of the contralateral breast.
- 5.8 No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, intraductal or lobular carcinoma *in situ* of the breast (diagnosed at any time), or any other cancer from which the patient has been disease-free for 5 years. Bilateral breast cancer patients are not eligible except for synchronous (diagnosed at the same time, i.e., within four weeks of initial histologic diagnosis) carcinoma of the contralateral breast, provided neither tumor is N3 or T4, one or both sides have \geq 4 nodes involved, and either a modified radical mastectomy or breast sparing surgery plus axillary node dissection (meeting the criteria in Section 5.2) has been performed for both tumors.
- 5.9 Patients must not have serious medical or psychiatric illness which prevents informed consent or participation in this trial.
- 5.10 All patients must have been evaluated and approved for this study by a transplant center approved for this study by one of the participating cooperative groups (see Section 19.4). This evaluation includes confirmation of bed availability at the transplant center and confirmation that a written request for insurance approval has been submitted (and/or confirmation of ability to pay for transplant). This evaluation must take place prior to registration.
- 5.11 **Effective 9/15/00:** If the patient is being registered by a Southwest Oncology Group institution, the patient must also be registered to the companion hematopoiesis protocol, **S9719**, at the time of registration to **S9623**, within the same registration phone call, unless the patient refuses to give consent for **S9719**. (See **S9719** for registration information.)
- 5.12 Pregnant or nursing women may not participate. Men are ineligible. Women of child-bearing potential must be planning to use effective contraception.
- 5.13 All patients must be informed of the investigational nature of this study and give written informed consent in accordance with institution and federal guidelines.
- 5.14 At the time of registration, the date of institutional review board approval for this study must be provided to the Statistical Center.

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

- 10.1 Disease-free survival: From date of initial randomization to date relapse or death is first documented.
- 10.2 Overall survival: Survival is measured from the date of randomization to the date of death.
- 10.3 Relapse: Appearance of any new lesions during or after protocol treatment. Whenever possible, relapses should be documented histologically.
- 10.4 **Performance Status:** Patients will be graded according to the current Southwest Oncology Group grading scale:

<u>GRADE</u>	<u>SCALE</u>
0	Fully active; able to carry on all predisease activities without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

11.0 STATISTICAL CONSIDERATIONS

- 11.1 *(original)* Accrual is expected to be greater than that on **SWOG-9114** (CALGB managed intergroup study in patients with 10+ nodes), more than 200 patients per year.
- (revised)* Accrual in 4 - 9 node positive patients has decreased to approximately 80 per year since May 1999. It is anticipated the addition of 10+ node patients will add another 120 per year.
- 11.2 *(original)* The primary question of interest is whether disease-free survival (DFS) is superior on the transplant arm. Assuming exponential DFS distributions and 60% five year DFS on the Arm A, then 5 years of accrual (500 eligible patients per arm) and 2 additional years of follow-up are required for a one-sided .025 level test to have power .9 to detect a hazard ratio of 1.45. (For exponential distributions this corresponds to a 45% improvement in median DFS due to the transplant arm, or to a difference of .6 vs .7 in five year DFS). All patients will be analyzed according to the assigned arm, including those on the transplant arm who do not receive transplant.
- (revised)* The primary question of interest is whether disease-free survival (DFS) is superior on the transplant arm. Approximately 350 events are required for a one-sided .025 level test to have power .9 to detect a hazard ratio of 1.45. The event rate for 4 - 9 node positive patients has been lower than anticipated, so 1000 eligible patients is still

the accrual goal for the study. Assuming 75% 5 year DFS, 2 1/2 additional years of accrual and 3 years of followup will result in approximately 225 events (180 from the first 3 1/2 years of accrual, 45 from the next 2 1/2 years). Assuming 55% 5 year DFS for 10+ node positive patients, approximately 125 events will result from this group.

- 11.3 Two interim analyses will be performed after approximately one-third and two-thirds of the expected number of events have occurred. Consideration will be given to reporting the study early if either 1) the transplant arm is superior at the .005 level, or 2) results are inconsistent with a 45% improvement due to the transplant arm at the .005 level. The final analysis will be done at the one-sided .02 level to adjust for the interim analyses.
- 11.4 PBPC samples from S9623 patients randomized to the PBPC-supported arm will be analyzed for the presence of breast cancer. Assuming that 5-year disease-free survival for patients with tumor-positive PBPCs is 60% and that 30% will have tumor detected in the PBPCs, a one-sided .05 level test will have approximately 80% power to detect a hazard ratio of 2 with accrual of 250 patients over 3 years and 2 years of follow-up.

11.5 Data Monitoring Committee (DMC)

This study will be monitored throughout accrual and follow-up periods by the Southwest Oncology Group Data Monitoring Committee (DMC). There is a single DMC to monitor all Southwest Oncology Group Phase III therapeutic trials. This committee is responsible for reviewing interim analyses prepared by the study statistician and for recommending whether the study needs to be modified or terminated based on these analyses. This committee also determines when the study results will be submitted for publication or otherwise released to the public. It will review any major modifications to the study proposed by the Study Committee.

The Study Committee consists of the Study Coordinators, study Statistician, Discipline Coordinators and a representative from each participating Group for intergroup studies. The Study Committee is responsible for monitoring the data from the study for toxicity, feasibility and accrual. The study committee also initiates minor changes in the study such as clarification of eligibility criteria.

12.0 DISCIPLINE REVIEW

Discipline review is not required.

13.0 REGISTRATION GUIDELINES

- 13.1 Registration, Southwest Oncology Group Investigators: All patients must be registered with the Southwest Oncology Group Statistical Center by telephoning 206/667-4623, 6:30 a.m. to 5:00 p.m. Pacific time, Monday through Friday, excluding holidays. Patients must be registered prior to initiation of treatment no more than one working day prior to the planned start of treatment. No exceptions will be permitted.

NOTE: ALL Southwest Oncology Group Institutions must register patients to the companion protocol, S9719, at the same time as S9623 registration, within the same registration phone call, unless the patient refuses to give consent for S9719. (If the patient refuses to give consent for S9719, this refusal must be documented on the initial S9623 flow sheet.)

Registration, NCCTG Investigators: All patients will be registered by calling 507/284-4130, or Faxing a completed Eligibility Checklist (507/284-0885) to the NCCTG Randomization Center between 8:30 a.m. to 4:30 p.m., Central Time, Monday through Friday, excluding holidays. The NCCTG Randomization Center will obtain and confirm all eligibility criteria. The NCCTG office will then contact the Southwest Oncology Group Statistical Center to randomize the patient and will contact the institution to relay the treatment assignment. A confirmation of randomization will be forwarded to the institution through the NCCTG office. Patients must be registered prior to initiation of treatment no more than one working day prior to the planned start of treatment. No exceptions will be permitted.

Registration, ECOG Investigators: **A signed HHS 310 Form, a copy of the Institution's IRB-approved informed consent document, and written justification for any changes made to the informed consent for this protocol must be on file at the ECOG Coordinating Center before an ECOG Institutions may enter patients.** The signed HHS 310, institution informed consent, and investigators justification for changes will be submitted to the following address:

ECOG Coordinating Center
Frontier Science
Attn: IRB
303 Boylston Street
Brookline, MA 02146-7648

Patients must not start protocol treatment prior to registration.

Letter from NCI Director Klausner to Oncologists

May 19, 2000

Dear ASCO Members:

We at the National Cancer Institute applaud your emphasis on quality of care at this Year 2000 ASCO meeting. As you know, a major role of NCI is to help you define quality cancer care by supporting clinical trials that answer key questions regarding treatment options. Among the key questions for clinicians today, one of the most highly publicized remains the role of high dose chemotherapy with transplant for breast cancer.

The largest NCI-sponsored trial testing this question is still open, but is in dire need of your support. Led by the Southwest Oncology Group, trial S9623 targets women at high risk for recurrence with four or more positive lymph nodes. This trial tests whether high-dose chemotherapy with transplant is superior to an alternate approach that uses G-CSF to support sequential use of high-dose chemotherapy that does not require transplant. Results from this trial will have important implications for both patients and physicians. The trial needs 1,000 patients and so far has accrued just over 500. Ever since the ASCO 1999 meeting when preliminary results from several transplant trials did not show a survival advantage, enrollment has suffered. If accrual does not pick up, then results from this trial, originally anticipated in 2002, will not be available for many more years. Importantly, this trial tests the question in a different subset of patients than did prior NCI-sponsored trials, and accordingly the preliminary results of these earlier trials may not be applicable to this subset.

Phase II trial results of high-dose therapy with transplant support in the advanced disease setting showed promising complete response rates. Based on these data, the NCI sponsored a series of national, randomized phase III trials beginning in 1990. One of these trials, led by the Eastern Cooperative Oncology Group (ECOG), has been published and did not reveal a benefit in metastatic disease. Preliminary results from a second, an adjuvant trial in women with ten or more positive lymph nodes led by the Cancer and Leukemia Group B, were presented at ASCO last year, and an update is anticipated this fall. This trial did show that those receiving transplant died less frequently of breast cancer, although this positive effect was counterbalanced by an increase in treatment-related deaths at the time of this initial presentation. A third trial in the same population has finished accrual and results from this ECOG study should be available in 2001.

Two other trials from Europe (Scandinavian and French) presented at ASCO last year also did not show a survival benefit, yet interpretation of their results is hampered by trial design and sample size constraints,

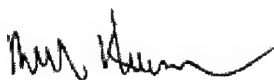
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respectively. In aggregate, the sum total of patients undergoing transplant in U.S. and international trials is rather unimpressive. Additional data is sorely needed. ASCO members will readily appreciate that it has taken many years and many thousands of patients to prove the effectiveness of systemic therapy in specific subsets of patients with adult solid tumors.

Emotions regarding transplant continue to run high. The question has been hotly debated in courtrooms, in legislatures, and especially in the media. Sentiment among patients and physicians shifts rapidly as each piece of new data become available. A positive trial from South Africa was recently negated when evidence of seriously fraudulent data was detected. Some have equated appropriate condemnation of this trial by the medical community with condemnation of transplant. Yet, the rationale behind the treatment approach remains sound. While there are experts who feel existing data suggest that ongoing trials will be negative, others are more optimistic. The only way to resolve this disagreement is through participation in large, carefully monitored clinical trials.

Better therapies are desperately needed for women at high risk of recurrence. Whether or not high-dose chemotherapy is a useful therapy for such women can only be determined if clinical trials testing this question appropriately are successfully completed. Your participation and support of S9623 is vital if important progress is to be made.

Sincerely yours,



Richard Klausner, M.D.
Director, National Cancer Institute

date posted 05/19/00

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NCI HIGH-PRIORITY CLINICAL TRIAL--Phase III Randomized Study of Intensive Sequential Doxorubicin, Paclitaxel, and Cyclophosphamide Versus Doxorubicin and Cyclophosphamide Followed By STAMP I or STAMP V Combination Chemotherapy With Autologous Stem Cell Rescue in Women With Primary Breast Cancer and At Least 4 Involved Axillary Lymph Nodes (Summary Last Modified 07/2000)

Patient Abstract

Rationale: Drugs used in chemotherapy use different ways to stop tumor cells from dividing so they stop growing or die. Peripheral stem cell transplantation may allow the doctor to give higher doses of chemotherapy drugs and kill more tumor cells. It is not yet known which regimen of chemotherapy followed by peripheral stem cell transplantation is more effective for breast cancer.

Purpose: Randomized phase III trial to compare the effectiveness of combination chemotherapy plus peripheral stem cell transplantation in treating women who have undergone surgery for breast cancer.

Eligibility:

- * At least four involved lymph nodes in the breast and/or armpit
- * No metastatic cancer
- * No more than 12 weeks since surgery
- * Must be enrolled on clinical trial SWOG-S9719
- * No previous chemotherapy
- * No previous hormone therapy or radiation therapy for breast cancer

Treatment: Patients will be randomly assigned to one of two groups. Patients in group one will receive infusions of doxorubicin once a week in weeks 1, 3, and 5, a 24-hour infusion of paclitaxel in weeks 7, 9, and 11, and infusions of cyclophosphamide in weeks 13, 15, and 17. They will also receive injections of filgrastim periodically during chemotherapy. Patients in group two will receive infusions of doxorubicin and cyclophosphamide in weeks 1, 4, 7, and 10. Bone marrow or peripheral stem cells will be collected. Patients will then receive high-dose combination chemotherapy for 4 days followed 3-4 days later with reinfusion of bone marrow or stem cells. Some patients may receive tamoxifen once a day for 5 years. Some patients may undergo radiation therapy 5 days a week for up to 5.5 weeks. Patients will receive follow-up evaluations every 4 months for 3 years, every 6 months for 2 years, and once a year thereafter.

This abstract is intended to give a brief overview of this clinical trial. To help determine whether the trial is appropriate for an individual, selected major eligibility criteria are listed above. To obtain more details related to trial eligibility and the treatment plan, please see the Health Professional abstract of this clinical trial. For more information about clinical trials, please visit the NCI cancerTrials website at <http://cancertrials.nci.nih.gov>.

Protocol IDs: SWOG-S9623, SWOG-9623

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Results of Omnibus Study with Women Ages 35-74 on Autologous Bone Marrow Transplants with High Dose Chemotherapy

Introduction and Method

From April 28 - May 9, 2000, the NCI and the National Alliance of Breast Cancer Organizations conducted a telephone omnibus survey with a nationally representative sample of 925 women ages 35-74.¹ The purpose of the study was to determine attitudes and perceptions of women toward high-dose chemotherapy with autologous bone marrow transplant (HDC-ABMT) as a treatment for breast cancer. The sample was 75% White, 11% Black, 8% Hispanic, and 4% other.

Description of Sample

- Ten percent of the sample reported that they had "ever been diagnosed and treated for cancer." Of that number, slightly over one quarter (26%) had been treated for breast cancer.
- Almost 4 in 10 respondents who had not themselves been diagnosed with breast cancer reported that they had a close family member or close friend that had "ever been diagnosed with breast cancer."
- Altogether, 43% of the sample had been "touched by cancer" (i.e., had either had cancer themselves or had a close family member/friend with breast cancer), and 55% were "untouched by cancer."

Key Findings

- Of the entire sample, almost half (46%) indicated that they had heard of using ABMT with chemotherapy as a treatment for breast cancer. As age increased, so did awareness of ABMT. Women ages 65-74 were significantly more likely to be aware of this treatment than the youngest women surveyed, ages 35-44 (58% vs. 40%).
- Of women who had heard of this treatment (n=435), 58% said they had either read or heard news reports over the past year regarding ABMT as a breast cancer treatment. Among this smaller group (n=262), two-thirds (67%) remembered these reports as being favorable toward the procedure. Interestingly, those "touched by cancer," and particularly those with a history of breast cancer were significantly less likely (60% and 32%, respectively) than those "untouched by cancer" (74%) to recall the reports as being positive in nature. Those who had had breast cancer themselves were also much more likely than those "untouched by cancer" to say that they could not remember (or

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didn't know) whether the reports were favorable or not (51% vs. 10%).

- When those who had heard about ABMT with chemotherapy to treat breast cancer (n=435) were asked whether they would consider this treatment if they were facing a decision about breast cancer treatment for themselves, half (50%) said they would be "very likely" to do so, and another 28% said they would be "somewhat likely." Again, age was a key factor for consideration of ABMT. The youngest women surveyed (ages 35-44) were much more likely than the oldest women surveyed (ages 65-74) to say they would be "very likely" to consider the treatment (63% vs. 36%).
- The most frequently cited reasons expressed by those who said they were unlikely to consider ABMT with chemotherapy for themselves (n=58) were:

Don't know enough about it; need more information 17%
Consider treatment too toxic/harsh/risky 13%
Heard treatment doesn't work for breast cancer 11%
Prefer another treatment 11%
Don't like/trust experimental treatments 9%

- Of those who said they would consider ABMT with chemotherapy as a treatment option (n=336), almost half (48%) indicated they would be "very likely" to consider it as part of a randomized research study, if cost and convenience were not an issue. Another third (34%) said they would be "somewhat likely" to do so.
- Somewhat lower percentages said they would be "very likely" (29%) or "somewhat likely" (37%) to consider having ABMT with chemotherapy outside of a research study. However, it should be pointed out that these numbers may be artifactual, given that respondents had already expressed a rather strong preference for a randomized study in the previous question.

Conclusion

Survey results indicate that women ages 35-74 persist in the belief that high-dose chemotherapy with autologous bone marrow transplant is of value for the treatment of breast cancer. Not only were a sizeable number of women aware of the procedure, but many said they would be receptive to this treatment option if they had breast cancer themselves, and would consider having the procedure as part of a randomized clinical trial. These findings are of particular interest given the negative media reports concerning HDC-ABMT in the past year. Notwithstanding these reports, the public appears to be supportive of HDC-ABMT clinical trials.

¹ In an omnibus survey, interested organizations can insert or "piggy-back" their questions onto national, regularly scheduled weekly or twice-weekly surveys conducted by private research firms. The current omnibus was fielded in three waves by International Communications Research (ICR) in Media, PA. Data were weighted to provide nationally representative and projectable estimates of adult women ages 35-74 in households with telephones. The error interval is plus or minus 3.2 at the 95 % level of confidence.

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**STEM CELL DAMAGE NOT DETECTED POST CHEMO-
THERAPY FOR BREAST CANCER: PRELIMINARY DATA**

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Adjuvant therapy with anthracycline-based combination chemotherapy for patients with breast cancer has been shown to improve disease-free and overall survival. Unfortunately, therapy-related myelodysplasia (t-AML) or acute myeloid leukemia (t-AML) has emerged as an uncommon but well-established complication of adjuvant therapy for breast cancer using dose-intensive regimens. t-MDS/AML evolve as a result of expansion of an abnormal clone of hematopoietic stem cells which have acquired somatic mutations conferring a growth advantage. The development of clonal hematopoiesis may be one of the earliest events that occur in an evolving neoplastic process. The goal of our pilot study is to determine prospectively whether two different dose-intensive adjuvant regimens for breast cancer can induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis. We have developed two PCR-based complementary assays to detect clonality. The HUMARA (human androgen receptor assay) is a general clonality assay based on the detection of a polymorphism within the androgen receptor gene located on chromosome X. Microsatellite instability assays detect genetic damage resulting from defective DNA replication/repair mechanisms and reflect the presence of an altered clonal hematopoietic population. These assays will be prospectively performed on sequentially obtained blood and apheresis samples (peripheral blood stem cell collection) from 200 women enrolled on a single, randomized dose-intensive Southwest Oncology Group adjuvant breast cancer study for women with four to nine positive nodes (S9623), "A Comparison of Intensive Sequential Chemotherapy using Doxorubicin plus Paclitaxel plus Cyclophosphamide with High Dose Chemotherapy and Autologous Hematopoietic Progenitor Cell Support for Primary Breast Cancer in Women with 4-9 Involved Axillary Lymph Nodes, Phase III, Intergroup"). To date, samples from 21 women have been analyzed at specified time points during and following treatment. The HUMARA and microsatellite instability assays provide reproducible and complementary results. No clonality or microsatellite instability has been noted in the initial cohort of patients studied. With limited follow-up, neither regimen appears to initiate genetic damage that could result in development of hematologic malignancies.

The U.S. Army Medical Research and Materiel Command under DAMD17-97-1-7088 support this work.

Lay/Public Abstract

High dose chemotherapy for patients with breast cancer improves disease-free and overall survival. Unfortunately, an uncommon but well-established late complication in women who have received dose-intensive regimens for breast cancer is development of a second cancer, therapy-related myelodysplasia (t-MDS) or acute myeloid leukemia (t-AML). t-MDS/AML results from an expanded, abnormal clone of cells derived from an early progenitor blood cell that has acquired damage to its genetic makeup. The genetic (damage) changes to this primitive blood cell may provide a growth advantage, resulting in the emergence of an abnormal population of blood cells with the same genetic makeup as the initial damaged blood cell (clonal hematopoiesis). Clonal hematopoiesis may be an early step in the development of blood disorders like myelodysplasia and acute leukemia. We have used two complementary assays, using blood samples obtained at specified time points during and following breast cancer treatment, to detect the presence of clonal hematopoiesis, resulting from damage to bone marrow cells, that may be caused by intensive chemotherapy for high-risk breast cancer. The goal of our pilot study is to determine prospectively whether certain intensive treatment regimens designed to prevent the recurrence of breast cancer can actually induce genetic damage to hematopoietic stem cells, defined by the emergence of clonal hematopoiesis. With limited follow-up, neither regimen (repetitive cycles of dose-intensive combination chemotherapy vs. high-dose chemotherapy with autologous stem cell transplantation) appears to initiate genetic damage that could result in emergence of a clonal hematopoietic population and subsequent development of a hematologic malignancy.

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